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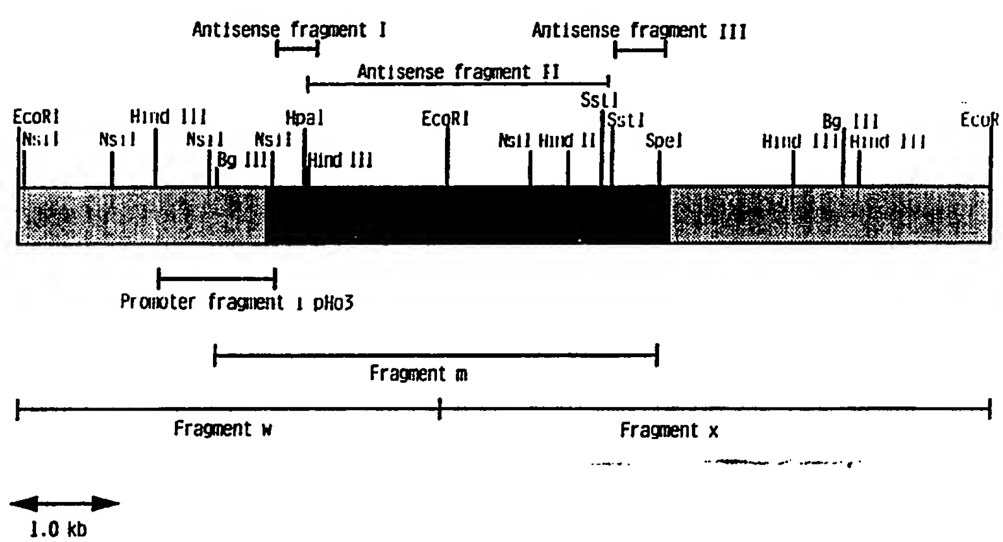
Result of restriction analysis. GBSS coding region including introns are marked in a darker tone.



(57) Abstract

Genetically engineered modification of potato for suppressing the formation of amylose-type starch is described. Three fragments for insertion in the antisense direction into the potato genome are also described. Moreover, antisense constructs, genes and vectors comprising said antisense fragments are described. Further a promoter for the gene coding for formation of granule-bound starch synthase and also the gene itself are described. Also cells, plants, tubers, microtubers and seeds of potato comprising said antisense fragments are described. Finally, amylopectin-type starch, both native and derivatised, derived from the potato that is modified in a genetically engineered manner, as well as a method of suppressing amylose formation in potato are described.

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GENETICALLY ENGINEERED MODIFICATION OF POTATO TO FORM AMYLOPECTIN-TYPE STARCH

The present invention relates to genetically engineered modification of potato, resulting in the formation of practically solely amylopectin-type starch in the potato. The genetically engineered modification implies the insertion of gene fragments into potato, said gene fragments comprising parts of leader sequence, translation start, translation end and trailer sequence as well as coding and noncoding (i.e. exons and introns) parts of the gene for granule-bound starch synthase, inserted in the antisense direction.

Background of the Invention

Starch in various forms is of great import in the food and paper industry. In future, starch will also be a great potential for producing polymers which are degradable in nature, e.g. for use as packing material. Many different starch products are known which are produced by derivatisation of native starch originating from, inter alia, maize and potato. Starch from potato and maize, respectively, is competing in most market areas.

In the potato tuber, starch is the greatest part of the solid matter. About 1/4 to 1/5 of the starch in potato is amylose, while the remainder of the starch is amylopectin. These two components of the starch have different fields of application, and therefore the possibility of producing either pure amylose or pure amylopectin is most interesting. The two starch components can be produced from common starch, which requires a number of process steps and, consequently, is expensive and complicated.

It has now proved that by genetic engineering it is possible to modify potato so that the tubers merely produce mainly starch of one or the other type. As a result, a starch quality is obtained which can compete in the areas where potato starch is normally not used today. Starch from such potato which is modified in a genetically

engineered manner has great potential as a food additive, since it has not been subjected to any chemical modification process.

Starch Synthesis

The synthesis of starch and the regulation thereof are presently being studied with great interest, both on the level of basic research and for industrial application. Although much is known about the assistance of certain enzymes in the transformation of saccharose into starch, the biosynthesis of starch has not yet been elucidated. By making researches above all into maize, it has, however, been possible to elucidate part of the ways of synthesis and the enzymes participating in these reactions. The most important starch-synthesising enzymes for producing the starch granules are the starch synthase and the branching enzyme. In maize, three forms of starch synthase have so far been demonstrated and studied, two of which are soluble and one is insolubly associated with the starch granules. Also the branching enzyme consists of three forms which are probably coded by three different genes (Mac Donald & Preiss, 1985; Preiss, 1988).

The Waxy Gene in Maize

The synthesis of the starch component amylose essentially occurs by the action of the starch synthase alpha-1,4-D-glucane-4-alpha-glucosyl transferase (EC 2.4.1.21) which is associated with the starch granules in the growth cell. The gene coding for this granule-bound enzyme is called "waxy" (= wx^+), while the enzyme is called "GBSS" (granule-bound starch synthase).

waxy locus in maize has been thoroughly characterised both genetically and biochemically. The waxy gene on chromosome 9 controls the production of amylose in endosperm, pollen and the embryo sac. The starch formed in endosperm in normal maize with the wx^+ allele consists to 25% of amylose and to 75% of amylopectin. A mutant form of maize has been found in which the endosperm contains a mutation located to the wx^+ gene, and therefore no functioning GBS

is synthesised. Endosperm from this mutant maize therefore contains merely amylopectin as the starch component. This so-called waxy mutant thus contains neither GBSS nor amylose (Echt & Schwartz, 1981).

The GBSS protein is coded by the wx^+ gene in the cell nucleus but is transported to and active in the amyloplast. The preprotein therefore consists of two components, viz. a 7 kD transit peptide which transfers the protein across the amyloplast membrane, and the actual protein which is 58 kD. The coding region of the wx^+ gene in maize is 3.7 kb long and comprises 14 exons and 13 introns. A number of the regulation signals in the promoter region are known, and two different polyadenylating sequences have been described (Klösgen et al, 1986; Schwartz-Sommer et al, 1984; Shure et al, 1983).

<u>Amylose Enzyme in Potato</u>

In potato, a 60 kD protein has been identified, which constitutes the main granule-bound protein. Since antibodies against this potato enzyme cross-react with GBSS from maize, it is assumed that it is the granule-bound synthase (Vos-Scheperkeuter et al, 1986). The gene for potato GBSS has, however, so far not been characterised to the same extent as the waxy gene in maize, either in respect of locating or structure.

Naturally occurring waxy mutants have been described for barley, rice and sorghum besides maize. In potato no natural mutant has been found, but a mutant has been produced by X-radiation of leaves from a monohaploid (n=12) plant (Visser et al, 1987). Starch isolated from tubers of this mutant contains neither the GBSS protein nor amylose. The mutant is conditioned by a simple recessive gene and is called amf. It may be compared to waxy mutants of other plant species since both the GBSS protein and amylose are lacking. The stability of the chromosome number, however, is weakened since this is quadrupled to the natural number (n=48), which can give negative effects on the potato plants (Jacobsen et al, 1990).

Inhibition of Amylose Production

The synthesis of amylose can be drastically reduced by inhibition of the granule-bound starch synthase, GBSS, which catalyses the formation of amylose. This inhibition results in the starch mainly being amylopectin.

Inhibition of the formation of enzyme can be accomplished in several ways, e.g. by:

- mutagen treatment which results in a modification of the gene sequence coding for the formation of the enzyme
- incorporation of a transposon in the gene sequence coding for the enzyme
- genetically engineered modification so that the gene coding for the enzyme is not expressed, e.g. antisense gene inhibition.

Fig. 1 illustrates a specific suppression of normal gene expression in that a complementary antisense nucleotide is allowed to hybridise with mRNA for a target gene. The antisense nucleotide thus is antisense RNA which is transcribed in vivo from a "reversed" gene sequence (Izant, 1989).

By using the antisense technique, various gene functions in plants have been inhibited. The antisense construct for chalcone synthase, polygalacturonase and phosphinotricin acetyltransferase has been used to inhibit the corresponding enzyme in the plant species petunia, tomato and tobacco.

Inhibition of Amylose in Potato

In potato, experiments have previously been made to inhibit the synthesis of the granule-bound starch synthase (GBSS protein) with an antisense construct corresponding to the gene coding for GBSS (this gene is hereinafter called the "GBSS gene"). Hergersberger (1988) describes a method by which a cDNA clone for the GBSS gene in potato has been isolated by means of a cDNA clone for the wx^+ gene in maize. An antisense construct based on the entire cDNA clone was transferred to leaf discs of potato by means of Agrobacterium tumefaciens. In microtubers induce

in vitro from regenerated potato sprouts, a varying and very weak reduction of the amylose content was observed and shown in a diagram. A complete characterisation of the GBSS gene is not provided.

The gene for the GBSS protein in potato has been further characterised in that a genomic wx^+ clone was examined by restriction analysis. However, the DNA sequence of the clone has not been determined (Visser et al, 1989).

Further experiments with an antisense construct corresponding to the GBSS gene in potato have been reported. The antisense construct which is based on a cDNA clone together with the CaMV 35S promoter has been transformed by means of Agrobacterium rhizogenes. According to information, the transformation resulted in a lower amylose content in the potato, but no values have been accounted for (Flavell, 1990).

None of the methods used so far for genetically engineered modification of potato has resulted in potato with practically no amylose-type starch.

The object of the invention therefore is to provide a practically complete suppression of the formation of amylose in potato tubers.

Summary of the Invention

According to the invention, the function of the GBSS gene and, thus, the amylose production in potato are inhibited by using completely new antisense constructs. For forming the antisense fragments according to the invention, the genomic GBSS gene is used as a basis in order to achieve an inhibition of GBSS and, consequently, of the amylose production, which is as effective as possible. The antisense constructs according to the invention comprise both coding and noncoding parts of the GBSS gene which correspond to sequences in the region comprising promoter as well as leader sequence, translation start, translation end and trailer sequence in the antisense direction. For a tissue-specific expression, i.e. the amylose production should be inhibited in the potato tubers only, use is made

of promoters which are specifically active in the potato tuber. As a result, the starch composition in other parts of the plant is not affected, which otherwise would give negative side-effects.

The invention thus comprises a fragment which essentially has one of the nucleotide sequences stated in SEQ ID No. 1, SEQ ID No. 2 or SEQ ID No. 3. However, the sequences may deviate from those stated by one or more non-adjacent base pairs, without affecting the function of the fragments.

The invention also comprises a potato-tuber-specific promoter comprising 987 bp which belongs to the gene according to the invention, which codes for granule-bound starch synthase. Neither the promoter nor the corresponding gene has previously been characterised. The promoter sequence of 987 bp is stated in SEQ ID No. 4, while the gene sequence is stated in SEQ ID No. 5. Also the promoter and gene sequences may deviate from those stated by one or more non-adjacent base pairs, without affecting their function.

The invention also comprises vectors including the antisense fragments and the antisense constructs according to the invention.

In other aspects the invention comprises cells, plants, tubers, microtubers and seeds whose genome contains the fragments according to the invention inserted in the antisense direction.

In still further aspects, the invention comprises amylopectin-type starch, both native and derivatised.

Finally, the invention comprises a method of suppressing amylose formation in potato, whereby mainly amylopectin-type starch is formed in the potato.

The invention will now be described in more detail with reference to the accompanying Figures in which

Fig. 1 illustrates the principle of the antisense gene inhibition,

Fig. 2 shows the result of restriction analysis of the potato GBSS gene,

Fig. 3 shows two new binary vectors pHo3 and pHo4,

Fig. 4 shows the antisense constructs pHoxwA, pHoxwB and pHoxwD,

Fig. 5 shows the antisense constructs pHoxwF and pHoxwG, and

Fig. 6 shows the antisense constructs pHoxwK and pHoxwL.

Moreover, the sequences of the different DNA fragments according to the invention are shown in SEQ ID Nos 1, 2, 3, 4 and 5. There may be deviations from these sequences in one or more non-adjacent base pairs.

MATERIALS

In the practical carrying out of the invention the following materials were used:

Bacterial strains: E. coli DH5alfa and DH5alfaF'IQ(BRL). E. coli JM105 (Pharmacia). A. tumefaciens LBA4404 (Clontech).

Vectors: M13mp18 and mp19 (Pharmacia). pBI101 and pBI121 (Clontech). pBI240.7 (M. W. Bevan). pUC plasmids (Pharmacia).

Enzymes: Restriction enzymes and EcoRI linker (BRL). UNION™ DNA Ligation Kit (Clontech). Sequenase™ DNA Sequencing Kit (USB). T_4-DNA ligase (Pharmacia).

The above-mentioned materials are used according to specifications stated by the manufacturers.

Genomic Library

A genomic library in EMBL3 has been produced by Clontech on the applicant's account, while using leaves of the potato Bintje as starting material.

Identification and Isolation of the GBSS Gene

The genomic library has been screened for the potato GBSS gene by means of cDNA clones for both the 5' and 3' end of the gene (said cDNA clones being obtained from M Hergersberger, Max Plank Institute in Cologne) according to a protocol from Clontech.

A full-length clone of the potato GBSS gene, wx311, has been identified and isolated from the genomic library. The start of the GBSS gene has been determined at an EcoRI fragment which is called fragment w (3.95 kb). The end of the GBSS gene has also been determined at an EcoRI fragment which is called fragment x (5.0 kb). A BgIII-SpeI fragment which is called fragment m (3.9 kb) has also been isolated and shares sequences both from fragment w and from fragment x. The fragments w, m and x have been subcloned in pUC13 (Viera, 1982; Yanisch-Peron et al, 1985) and are called pSw, pSm and pSx, respectively (Fig. 2).

Characterisation of the GBSS Gene in Potato

The GBSS gene in potato has been characterised by restriction analysis and cDNA probes, where the 5' and 3' end of the GBSS gene has been determined more accurately (Fig. 2). Sequence determination according to Sanger et al, 1977 of the GBSS gene has been made on subclones from pSw and pSx in M13mp18 and mp19 as well as pUC19 starting around the 5' end (see SEQ ID No. 5).

The promoter region has been determined at a BglII-NsiI fragment (see SEQ ID No. 4). Transcription and translation start has been determined at an overlapping BglII-HindIII fragment. The terminator region has in turn been determined at a SpeI-HindIII fragment.

Antisense Constructs for the GBSS Gene in Potato

The GBSS gene fragments according to the invention (see SEQ ID Nos 1, 2 and 3, and Fig. 2) have been determined in the following manner.

The restriction of pSw with NsiI and HindIII gives fragment I (SEQ ID No. 1) which subcloned in pUC19 is called 19NH35. Further restriction of 19 NH35 with HpaI-SstI gives a fragment containing 342 bp of the GBSS gene according to the invention. This fragment comprises leader sequence, translation start and the first 125 bp of the coding region.

The restriction of pSm with HpaI and NsiI gives fragment II (SEQ ID No. 2) which subcloned in pJRD184 (Heusterspreute et al, 1987) is called pJRDmitt. Further restriction of pJRDmitt with HpaI-SstI gives a fragment containing 2549 bp of the GBSS gene according to the invention. This fragment comprises exons and introns from the middle of the gene.

The restriction of pSx with SstI and SpeI gives fragment III (SEQ ID No. 3) which subcloned in pBluescript (Melton et al, 1984) is called pBlue3'. Further restriction of pBlue3' with BamHI-SstI gives a fragment containing 492 bp of the GBSS gene according to the invention. This fragment comprises the last intron and exon, translation end and 278 bp of trailer sequence.

<u>Antisense Constructs with Fragment I (Fig. 4)</u>: For the antisense construct pHoxwA, the HpaI-SstI fragment from 19NH35 has been inserted in the antisense direction into the binary vector pBI121 (Jefferson et al, 1987) cleaved with SmaI-SstI. The transcription of the antisense fragment is then initiated by the CaMV 35S promoter and is terminated by the NOS terminator (NOS = nopaline synthase).

For the antisense construct pHoxwB, the HpaI-SstI fragment from 19NH35 has been inserted in the antisense direction into the binary vector pHo4 (Fig. 3) cleaved with SmaI-SstI. The patatin I promoter which is tuber specific in potato comes from the vector pBI240.7 obtained from M. Bevan, Institute of Plant Science Research, Norwich. The transcription of the antisense fragment is then initiated by the patatin I promoter and is terminated by the NOS terminator.

For the antisense construct pHoxwD, the HpaI-SstI fragment from 19NH35 has been inserted in the antisense direction into the binary vector pHo3 (Fig. 3) cleaved with SmaI-SstI. pHo3 is a new binary vector which is constructed on the basis of pBI101. This vector which contains the promoter according to the invention (see SEQ ID

No. 4) (GBSS promoter) of the now characterised potato GBSS gene according to the invention has been restriction-cleaved with SmaI and SstI, the HpaI-SstI fragment from 19NH35 being inserted in the antisense direction. The transcription of the antisense fragment is then initiated by its own GBSS promoter and is terminated by the NOS terminator. This means that the antisense fragment is transcribed only in the potato tuber, since the GBSS promoter like the patatin I promoter is tuber-specific.

Antisense Constructs with Fragment II (Fig. 5): For the antisense construct pHoxwF, the HpaI-SstI fragment from pJRDmitt has been inserted in the antisense direction into the binary vector pHo4 cleaved with SmaI-SstI. The transcription of the antisense fragment is then initiated by the patatin I promoter and terminated by the NOS terminator.

For the antisense construct pHoxwG, the HpaI-SstI fragment from pJRDmitt has been inserted in the antisense direction into the binary vector pHo3 cleaved with SmaI-SstI. The transcription of the antisense fragment is then initiated by its own GBSS promoter and is terminated by the NOS terminator.

Antisense Constructs with Fragment III (Fig. 6): For the antisense construct pHoxwK, the BamHI-SstI fragment from pBlue3' has been inserted in the antisense direction into the binary vector pHo4 cleaved with BamHI-SstI. The transcription of the antisense fragment is then initiated by the patatin I promoter and is terminated by the NOS terminator.

For the antisense construct pHoxwL, the BamHI-SstI fragment from pBlue3' has been inserted in the antisense direction into the binary vector pHo3 cleaved with BamHI-SstI. The transcription of the antisense fragment is then initiated by its own GBSS promoter and is terminated by the NOS terminator.

The formed antisense constructs (Figs 4, 5, 6) have been transformed to Agrobacterium tumefaciens strain LBA4404 by direct transformation with the "freeze-thawing" method (Hoekema et al, 1983; An et al, 1988).

Transformation

The antisense constructs are transferred to bacteria, suitably by the "freeze-thawing" method (An et al, 1988). The transfer of the recombinant bacterium to potato tissue occurs by incubation of the potato tissue with the recombinant bacterium in a suitable medium after some sort of damage has been inflicted upon the potato tissue. During the incubation, T-DNA from the bacterium enters the DNA of the host plant. After the incubation, the bacteria are killed and the potato tissue is transferred to a solid medium for callus induction and is incubated for growth of callus.

After passing through further suitable media, sprouts are formed which are cut away from the potato tissue.

Checks for testing the expression of the antisense constructs and the transfer thereof to the potato genome are carried out by e.g. southern and northern hybridisation (Maniatis et al (1982)). The number of copies of the antisense construct which has been transferred is determined by southern hybridisation.

The testing of the expression on protein level is suitably carried out on microtubers induced in vitro on the transformed sprouts, thus permitting the testing to be performed as quickly as possible.

Characterisation of the GBSS Protein

The effect of the antisense constructs on the function of the GBSS gene with respect to the activity of the GBSS protein is examined by extracting starch from the microtubers and analysing it regarding the presence of the GBSS protein. In electrophoresis on polyacrylamide gel (Hovenkamp-Hermelink et al, 1987), the GBSS protein forms a distinct band at 60 kD, when the GBSS gene functions. When the GBSS gene is not expressed, i.e. when the anti-

sense GBSS gene is fully expressed, thereby inhibiting the formation of GBSS protein, no 60 kD band is demonstrated on the gel.

Characterisation of the Starch

The composition of the starch in microtubers is identical with that of ordinary potato tubers, and therefore the effect of the antisense constructs on the amylose production is examined in microtubers. The proportion of amylose to amylopectin can be determined by a spectrophotometric method (e.g. according to Hovenkamp-Hermelink et al, 1988).

Extraction of Amylopectin from Amylopectin Potato

Amylopectin is extracted from the so-called amylopectin potato (potato in which the formation of amylose has been suppressed by inserting the antisense constructs according to the invention) in a known manner.

Derivatisation of Amylopectin

Depending on the final use of the amylopectin, its physical and chemical qualities can be modified by derivatisation. By derivatisation is here meant chemical, physical and enzymatic treatment and combinations thereof (modified starches).

The chemical derivatisation, i.e. chemical modification of the amylopectin, can be carried out in different ways, for example by oxidation, acid hydrolysis, dextrinisation, different forms of etherification, such as cationisation, hydroxy propylation and hydroxy ethylation, different forms of esterification, for example by vinyl acetate, acetic anhydride, or by monophosphatising, diphosphatising and octenyl succination, and combinations thereof.

Physical modification of the amylopectin can be effected by e.g. cylinder-drying or extrusion.

In enzymatic derivatisation, degradation (reduction of the viscosity) and chemical modification of the amylopectin are effected by means of existing enzymatic systems.

The derivatisation is effected at different temperatures, according to the desired end product. The ordinary range of temperature which is used is 20-45°C, but temperatures up to 180°C are possible.

The invention will be described in more detail in the following Examples.

Example 1

Production of microtubers with inserted antisense constructs according to the invention

The antisense constructs (see Figs 4, 5 and 6) are transferred to Agrobacterium tumefaciens LBA 4404 by the "freeze-thawing" method (An et al, 1988). The transfer to potato tissue is carried out according to a modified protocol from Rocha-Sosa et al (1989).

Leaf discs from potato plants cultured in vitro are incubated in darkness on a liquid MS-medium (Murashige & Skoog; 1962) with 3% saccharose and 0.5% MES together with 100 µl of a suspension of recombinant Agrobacterium per 10 ml medium for two days. After these two days the bacteria are killed. The leaf discs are transferred to a solid medium for callus induction and incubated for 4-6 weeks, depending on the growth of callus. The solid medium is composed as follows:

MS + 3% saccharose

2 mg/l	zeatin riboside
0.02 mg/l	"NAA"
0.02 mg/l	"GA_3"
500 mg/l	"Claforan"
50 mg/l	kanamycin
0.25%	"Gellan"

Subsequently the leaf discs are transferred to a medium having a different composition of hormones, comprising:

MS + 3% saccharose
5 mg/l "NAA"
0.1 mg/l "BAP"
500 mg/l "Claforan"
50 mg/l kanamycin
0.25% "Gellan"

The leaf discs are stored on this medium for about 4 weeks, whereupon they are transferred to a medium in which the "Claforan" concentration has been reduced to 250 mg/l. If required, the leaf discs are then moved to a fresh medium every 4 or 5 weeks. After the formation of sprouts, these are cut away from the leaf discs and transferred to an identical medium.

The condition that the antisense construct has been transferred to the leaf discs is first checked by analysing leaf extracts from the regenerated sprouts in respect of glucuronidase activity by means of the substrates described by Jefferson et al (1987). The activity is demonstrated by visual assessment.

Further tests of the expression of the antisense constructs and the transfer thereof to the potato genome are carried out by southern and northern hybridisation according to Maniatis et al (1981). The number of copies of the antisense constructs that has been transferred is determined by southern hybridisation.

When it has been established that the antisense constructs have been transferred to and expressed in the potato genome, the testing of the expression on protein level begins. The testing is carried out on microtubers which have been induced in vitro on the transformed sprouts, thereby avoiding the necessity of waiting for the development of a complete potato plant with potato tubers.

Stem pieces of the potato sprouts are cut off at the nodes and placed on a modified MS medium. There they form microtubers after 2-3 weeks in incubation in darkness at 19°C (Bourque et al, 1987). The medium is composed as follows:

MS + 6% saccharose
2.5 mg/l kinetin
2.5 mg/l "Gellan"

The effect of the antisense constructs on the function of the GBSS gene in respect of the activity of the GBSS protein is analysed by means of electrophoresis on polyacrylamide gel (Hovenkamp-Hermelink et al, 1987). Starch is extracted from the microtubers and analysed regarding the presence of the GBSS protein. In a polyacrylamide gel, the GBSS protein forms a distinct band at 60 kD, when the GBSS gene functions. If the GBSS gene is not expressed, i.e. when the antisense GBSS gene is fully expressed so that the formation of GBSS protein is inhibited, no 60 kD band can be seen on the gel.

The composition of the starch, i.e. the proportion of amylose to amylopectin, is determined by a spectrophotometric method according to Hovenkamp-Hermelink et al (1988), the content of each starch component being determined on the basis of a standard graph.

Example 2

Extraction of amylopectin from amylopectin potato.

Potato whose main starch component is amylopectin, below called amylopectin potato, modified in a genetically engineered manner according to the invention, is grated, thereby releasing the starch from the cell walls.

The cell walls (fibres) are separated from fruit juice and starch in centrifugal screens (centrisiler). The fruit juice is separated from the starch in two steps, viz. first in hydrocyclones and subsequently in specially designed band-type vacuum filters.

Then a finishing refining is carried out in hydrocyclones in which the remainder of the fruit juice and fibres are separated.

The product is dried in two steps, first by predrying on a vacuum filter and subsequently by final drying in a hot-air current.

Example 3

Chemical derivatisation of amylopectin

Amylopectin is sludged in water to a concentration of 20-50%. The pH is adjusted to 10.0-12.0 and a quatenary ammonium compound is added in such a quantity that the end product obtains a degree of substitution of 0.004-0.2. The reaction temperature is set at 20-45°C. When the reaction is completed, the pH is adjusted to 4-8, whereupon the product is washed and dried. In this manner the cationic starch derivative 2-hydroxy-3-trimethyl ammonium propyl ether is obtained.

Example 4

Chemical derivatisation of amylopectin

Amylopectin is sludged in water to a water content of 10-25% by weight. The pH is adjusted to 10.0-12.0, and a quatenary ammonium compound is added in such a quantity that the end product obtains a degree of substitution of 0.004-0.2. The reaction temperature is set at 20-45°C. When the reaction is completed, the pH is adjusted to 4-8. The end product is 2-hydroxy-3-trimethyl ammonium propyl ether.

Example 5

Chemical derivatisation of amylopectin

Amylopectin is sludged in water to a concentration of 20-50% by weight. The pH is adjusted to 5.0-12.0, and sodium hypochlorite is added so that the end product obtains the desired viscosity. The reaction temperature is set at 20-45°C. When the reaction is completed, the pH is adjusted to 4-8, whereupon the end product is washed and dried. In this manner, oxidised starch is obtained.

Example 6

Physical derivatisation of amylopectin

Amylopectin is sludged in water to a concentration of 20-50% by weight, whereupon the sludge is applied to a heated cylinder where it is dried to a film.

Example 7

Chemical and physical derivatisation of amylopectin

Amylopectin is treated according to the process described in one of Examples 3-5 for chemical modification and is then further treated according to Example 6 for physical derivatisation.

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SEQ ID No. 1

Sequenced molecule: genomic DNA

Name: GBSS gene fragment from potato

Length of sequence: 342 bp

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TGCATGTTTC CCTACATTCT ATTTAGAATC GTGTTGTGGT GTATAAACGT        50
TGTTTCATAT CTCATCTCAT CTATTCTGAT TTTGATTCTC TTGCCTACTG       100
TAATCGGTGA TAAATGTGAA TGCTTCCTTT CTTCTCAGAA ATCAATTTCT       150
GTTTTGTTTT TGTTCATCTG TAGCTTATTC TCTGGTAGAT TCCCCTTTTT       200
GTAGACCACA CATCAC ATG GCA AGC ATC ACA GCT TCA CAC CAC        243
                  Met Ala Ser Ile Thr Ala Ser His His
                   1               5

TTT GTG TCA AGA AGC CAA ACT TCA CTA GAC ACC AAA TCA ACC      285
Phe Val Ser Arg Ser Gln Thr Ser Leu Asp Thr Lys Ser Thr
 10                 15                  20

TTG TCA CAG ATA GGA CTC AGG AAC CAT ACT CTG ACT CAC AAT      327
Leu Ser Gln Ile Gly Leu Arg Asn His Thr Leu Thr His Asn
     25                  30                  35

GGT TTA AGG GCT GTT                                          342
Gly Leu Arg Ala Val
         40
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SEQ ID No. 2

Sequenced molecule: genomic DNA

Name: GBSS gene fragment from potato

Length of sequence: 2549 bp

AAC AAG CTT GAT GGG CTC CAA TCA ACA ACT AAT ACT AAG GTA 42
Asn Lys Leu Asp Gly Leu Gln Ser Thr Thr Asn Thr Lys Val
45 50 55

ACA CCC AAG ATG GCA TCC AGA ACT GAG ACC AAG AGA CCT GGA 84
Thr Pro Lys Met Ala Ser Arg Thr Glu Thr Lys Arg Pro Gly
60 65 70

TGC TCA GCT ACC ATT GTT TGT GGA AAG GGA ATG AAC TTG ATC 126
Cys Ser Ala Thr Ile Val Cys Gly Lys Gly Met Asn Leu Ile
75 80

TTT GTG GGT ACT GAG GTT GGT CCT TGG AGC AAA ACT GGT GGA 168
Phe Val Gly Thr Glu Val Gly Pro Trp Ser Lys Thr Gly Gly
85 90 95

CTA GGT GAT GTT CTT GGT GGA CTA CCA CCA GCC CTT GCA 207
Leu Gly Asp Val Leu Gly Gly Leu Pro Pro Ala Leu Ala
100 105 110

GTAAGTCTTT CTTTCATTTG GTTACCTACT CATTCATTAC TTATTTTGTT 257
TAGTTAGTTT CTACTGCATC AGTCTTTTTA TCATTTAG GCC CGC GGA 304
Ala Arg Gly

CAT CGG GTA ATG ACA ATA TCC CCC CGT TAT GAC CAA TAC AAA 346
His Arg Val Met Thr Ile Ser Pro Arg Tyr Asp Gln Tyr Lys
115 120 125

GAT GCT TGG GAT ACT GGC GTT GCG GTT GAG GTACATCTTC 386
Asp Ala Trp Asp Thr Gly Val Ala Val Glu
130 135

CTATATTGAT ACGGTACAAT ATTGTTCTCT TACATTTCCT GATTCAAGAA 436
TGTGATCATC TGCAG GTC AAA GTT GGA GAC AGC ATT GAA ATT GTT 481
Val Lys Val Gly Asp Ser Ile Glu Ile Val
140 145

CGT TTC TTT CAC TGC TAT AAA CGT GGG GTT GAT CGT GTT TTT 523
Arg Phe Phe His Cys Tyr Lys Arg Gly Val Asp Arg Val Phe
150 155 160

GTT GAC CAC CCA ATG TTC TTG GAG AAA GTAAGCATAT 560
Val Asp His Pro Met Phe Leu Glu Lys
165 170

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TATGATTATG AATCCGTCCT GAGGGATACG CAGAACAGGT CATTTTGAGT      610
ATCTTTTAAC TCTACTGGTG CTTTTACTCT TTTAAG GTT TGG GGC AAA     658
                                        Val Trp Gly Lys
                                                    175

ACT GGT TCA AAA ATC TAT GGC CCC AAA GCT GGA CTA GAT TAT     700
Thr Gly Ser Lys Ile Tyr Gly Pro Lys Ala Gly Leu Asp Tyr
                180                     185

CTG GAC AAT GAA CTT AGG TTC AGC TTG TTG TGT CAA             736
Leu Asp Asn Glu Leu Arg Phe Ser Leu Leu Cys Gln
190                 195                     200

GTAAGTTAGT TACTCTTGAT TTTTATGTGG CATTTTACTC TTTTGTCTTT      786
AATCGTTTTT TTAACCTTGT TTTCTCAG GCA GCC CTA GAG GCA CCT      832
                               Ala Ala Leu Glu Ala Pro
                                               205

AAA GTT TTG AAT TTG AAC AGT AGC AAC TAC TTC TCA GGA CCA     874
Lys Val Leu Asn Leu Asn Ser Ser Asn Tyr Phe Ser Gly Pro
        210                     215                 220

TAT G      GTAATTAACA CATCCTAGTT TCAGAAAACT CCTTACTATA      918
Tyr G

TCATTGTAGG TAATCATCTT TATTTTGCCT ATTCCTGCAG GA GAG GAT      966
                                            ly Glu Asp
                                                   225

GTT CTC TTC ATT GCC AAT GAT TGG CAC ACA GCT CTC ATT CCT    1008
Val Leu Phe Ile Ala Asn Asp Trp His Thr Ala Leu Ile Pro
                230                     235

TGC TAC TTG AAG TCA ATG TAC CAG TCC AGA GGA ATC TAC TTG    1050
Cys Tyr Leu Lys Ser Met Tyr Gln Ser Arg Gly Ile Tyr Leu
240                 245                     250

AAT GCC AAG            GTAAAATTTC TTTGTATTCA CTCGATTGCA    1089
Asn Ala Lys
    255

CGTTACCCTG CAAATCAGTA AGGTTGTATT AATATATGAT AAATTTCACA     1139
TTGCCTCCAG GTT GCT TTC TGC ATC CAT AAC ATT GCC TAC CAA     1182
           Val Ala Phe Cys Ile His Asn Ile Ala Tyr Gln
                       260                     265

GGT CGA TTT TCT TTC TCT GAC TTC CCT CTT CTC AAT CTT CCT    1224
Gly Arg Phe Ser Phe Ser Asp Phe Pro Leu Leu Asn Leu Pro
        270                     275                 280

GAT GAA TTC AGG GGT TCT TTT GAT TTC ATT GAT GGG TAT        1263
Asp Glu Phe Arg Gly Ser Phe Asp Phe Ile Asp Gly Tyr
            285                     290

GTATTTATGC TTGAAATCAG ACCTCCAACT TTTGAAGCTC TTTTGATGCT     1313
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AGTAAATTGA GTTTTTAAAA TTTTGCAGAT ATGAG AAG CCT GTT AAG     1360
                                       Lys Pro Val Lys
                                       295

GGT AGG AAA ATC AAC TGG ATG AAG GCT GGG ATA TTA GAA TCA    1402
Gly Arg Lys Ile Asn Trp Met Lys Ala Gly Ile Leu Glu Ser
    300                 305                 310

CAT AGG GTG GTT ACA GTG AGC CCA TAC TAT GCC CAA GAA CTT    1444
His Arg Val Val Thr Val Ser Pro Tyr Tyr Ala Gln Glu Leu
        315                 320                 325

GTC TCT GCT GTT GAC AAG GGA GTT GAA TTG GAC AGT GTC CTT    1486
Val Ser Ala Val Asp Lys Gly Val Glu Leu Asp Ser Val Leu
            330                 335                 340

CGT AAG ACT TGC ATA ACT GGG ATT GTG AAT GGC ATG GAT ACA    1528
Arg Lys Thr Cys Ile Thr Gly Ile Val Asn Gly Met Asp Thr
                345                 350

CAA GAG TGG AAC CCA GCG ACT GAC AAA TAC ACA GAT GTC AAA    1570
Gln Glu Trp Asn Pro Ala Thr Asp Lys Tyr Thr Asp Val Lys
355                 360                 365

TAC GAT ATA ACC ACT    GTAAGATAAG ATTTTTCCGA CTCCAGTATA    1615
Tyr Asp Ile Thr Thr
    370

TACTAAATTA TTTTGTATGT TTATGAAATT AAAGAGTTCT TGCTAATCAA     1665
AATCTCTATA CAG GTC ATG GAC GCA AAA CCT TTA CTA AAG GAG     1708
               Val Met Asp Ala Lys Pro Leu Leu Lys Glu
                   375                 380

GCT CTT CAA GCA GCA GTT GGC TTG CCT GTT GAC AAG AAG ATC    1756
Ala Leu Gln Ala Ala Val Gly Leu Pro Val Asp Lys Lys Ile
    385                 390                 395

CCT TTG ATT GGC TTC ATC GGC AGA CTT GAG GAG CAG AAA GGT    1792
Pro Leu Ile Gly Phe Ile Gly Arg Leu Glu Glu Gln Lys Gly
        400                 405                 410

TCA GAT ATT CTT GTT GCT GCA ATT CAC AAG TTC ATC GGA TTG    1834
Ser Asp Ile Leu Ala Val Ala Ile His Lys Phe Ile Gly Leu
            415                 420                 425

GAT GTT CAA ATT GTA GTC CTT       GTAAGTACCA AATGGACTCA    1875
Asp Val Gln Ile Val Val Leu
                430

TGGTATCTCT CTTGTTGAGT TTACTTGTGC CGAAACTGAA ATTGACCTGC     1925
TACTCATCCT ATGCATCAG GGA ACT GGC AAA AAG GAG TTT GAG       1968
                     Gly Thr Gly Lys Lys Glu Phe Glu
                             435                 440
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CAG GAG ATT GAA CAG CTC GAA GTG TTG TAC CCT AAC AAA GCT 2010
Gln Glu Ile Glu Gln Leu Glu Val Leu Tyr Pro Asn Lys Ala
445 450

AAA GGA GTG GCA AAA TTC AAT GTC CCT TTG GCT CAC ATG ATC 2052
Lys Gly Val Ala Lys Phe Asn Val Pro Leu Ala His Met Ile
455 460 465

ACT GCT GGT GCT GAT TTT ATG TTG GTT CCA AGC AGA TTT GAA 2094
Thr Ala Gly Ala Asp Phe Met Leu Val Pro Ser Arg Phe Glu
470 475 480

CCT TGT GGT CTC ATT CAG TTA CAT GCT ATG CGA TAT GGA ACA 2136
Pro Cys Gly Leu Ile Gln Leu His Ala Met Arg Tyr Gly Thr
485 490 495

GTAAGAACCA GAAGAGCTTG TACCTTTTTA CTGAGTTTTT AAAAAAAGAA 2186
TCATAAGACC TTGTTTTCCA TCTAAAGTTT AATAACCAAC TAAATGTTAC 2236
TGCAGCAAGC TTTTCATTTC TGAAAATTGG TTATCTGATT TTAACGTAAT 2286
CACATGTGAG TCAG GTA CCA ATC TGT GCA TCG ACT GGT GGA CTT 2330
Val Pro Ile Cys Ala Ser Thr Gly Gly Leu
500 505

GTT GAC ACT GTG AAA GAA GGC TAT ACT GGA TTC CAT ATG GGA 2372
Val Asp Thr Val Lys Glu Gly Tyr Thr Gly Phe His Met Gly
510 515 520

GCC TTC AAT GTT GAA GTATGTGATT TTACATCAAT TGTGTACTTG 2417
Ala Phe Asn Val Glu
525

TACATGGTCC ATTCTCGTCT TGATATACCC CTTGTTGCAT AAACATTAAC 2467
TTATTGCTTC TTGAATTTGG TTAG TGC GAT GTT GTT GAC CCA GCT 2512
Cys Asp Val Val Asp Pro Ala
530

GAT GTG CTT AAG ATA GTA ACA ACA GTT GCT AGA GCT C 2549
Asp Val Leu Lys Ile Val Thr Thr Val Ala Arg Ala
535 540

SEQ ID No. 3

Sequenced molecule: genomic DNA

Name: GBSS gene fragment from potato

Length of sequence: 492 bp

```
                                                                   45
GAG CTC TCC TGG AAG   GTAAGTGTGA ATTTGATAAT TTGCGTAGGT
Glu Leu Ser Trp Lys
    565

                                                                   95
ACTTCAGTTT GTTGTTCTCG TCAGCACTGA TGGATTCCAA CTGGTGTTCT            127
TGCAG            GAA CCT GCC AAG AAA TGG GAG ACA TTG
                 Glu Pro Ala Lys Lys Trp Glu Thr Leu
                     570                 575

                                                                  169
CTA TTG GGC TTA GGA GCT TCT GGC AGT GAA CCC GGT GTT GAA
Leu Leu Gly Leu Gly Ala Ser Gly Ser Glu Pro Gly Val Glu
        580                 585                 590

                                                                  211
GGG GAA GAA ATC GCT CCA CTT GCC AAG GAA AAT GTA GCC ACT
Gly Glu Glu Ile Ala Pro Leu Ala Lys Glu Asn Val Ala Thr
            595                 600                 605

                                                                  257
CCT TAA    ATGAGCTTTG GTTATCCTTG TTTCAACAAT AAGATCATTA
Pro ***
606

AGCAAACGTA TTTACTAGCG AACTATGTAG AACCCTATTA TGGGGTCTCA            307
ATCATCTACA AAATGATTGG TTTTTGCTGG GGAGCAGCAG CATATAAGGC            357
TGTAAAATCC TGGTTAATGT TTTTGTAGGT AAGGGCTATT TAAGGTGGTG            407
TGGATCAAAG TCAATAGAAA ATAGTTATTA CTAACGTTTG CAACTAAATA            457
CTTAGTAATG TAGCATAAAT AATACTAGAA CTAGT                            492
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SEQ ID No. 4

Sequenced molecule: genomic DNA

Name: Promoter for the GBSS gene from potato

Length of sequence: 987 bp

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AAGCTTTAAC GAGATAGAAA ATTATGTTAC TCCGTTTTGT TCATTACTTA     50
ACAAATGCAA CAGTATCTTG TACCAAATCC TTTCTCTCTT TTCAAACTTT    100
TCTATTTGGC TGTTGACGGA GTAATCAGGA TACAAACCAC AAGTATTTAA    150
TTGACTCCTC CGCCAGATAT TATGATTTAT GAATCCTCGA AAAGCCTATC    200
CATTAAGTCC TCATCTATGG ATATACTTGA CAGTATCTTC CTGTTTGGGT    250
ATTTTTTTTT CCTGCCAAGT GGAACGGAGA CATGTTATGA TGTATACGGG    300
AAGCTCGTTA AAAAAAAATA CAATAGGAAG AAATGTAACA AACATTGAAT    350
GTTGTTTTTA ACCATCCTTC CTTTAGCAGT GTATCAATTT TGTAATAGAA    400
CCATGCATCT CAATCTTAAT ACTAAAATGC AACTTAATAT AGGCTAAACC    450
AAGATAAAGT AATGTATTCA ACCTTTAGAA TTGTGCATTC ATAATTAGAT    500
CTTGTTTGTC GTAAAAAATT AGAAAATATA TTTACAGTAA TTTGGAATAC    550
AAAGCTAAGG GGGAAGTAAC TAATATTCTA GTGGAGGGAG GGACCAGTAC    600
CAGTACCTAG ATATTATTTT TAATTACTAT AATAATAATT TAATTAACAC    650
GAGACATAGG AATGTCAAGT GGTAGCGTAG GAGGGAGTTG GTTTAGTTTT    700
TTAGATACTA GGAGACAGAA CCGGACGGCC CATTGCAAGG CCAAGTTGAA    750
GTCCAGCCGT GAATCAACAA AGAGAGGGCC CATAATACTG TCGATGAGCA    800
TTTCCCTATA ATACAGTGTC CACAGTTGCC TTCTGCTAAG GGATAGCCAC    850
CCGCTATTCT CTTGACACGT GTCACTGAAA CCTGCTACAA ATAAGGCAGG    900
CACCTCCTCA TTCTCACTCA CTCACTCACA CAGCTCAACA AGTGGTAACT    950
TTTACTCATC TCCTCCAATT ATTTCTGATT TCATGCA                  987
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SEQ ID No. 5

Sequenced molecule: genomic DNA

Name: GBSS gene from potato

Length of sequence: 4964 bp

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AAGCTTTAAC GAGATAGAAA ATTATGTTAC TCCGTTTTGT TCATTACTTA      50
ACAAATGCAA CAGTATCTTG TACCAAATCC TTTCTCTCTT TTCAAACTTT     100
TCTATTTGGC TGTTGACGGA GTAATCAGGA TACAAACCAC AAGTATTTAA     150
TTGACTCCTC CGCCAGATAT TATGATTTAT GAATCCTCGA AAAGCCTATC     200
CATTAAGTCC TCATCTATGG ATATACTTGA CAGTATCTTC CTGTTTGGGT     250
ATTTTTTTTT CCTGCCAAGT GGAACGGAGA CATGTTATGA TGTATACGGG     300
AAGCTCGTTA AAAAAAAATA CAATAGGAAG AAATGTAACA AACATTGAAT     350
GTTGTTTTTA ACCATCCTTC CTTTAGCAGT GTATCAATTT TGTAATAGAA     400
CCATGCATCT CAATCTTAAT ACTAAAATGC AACTTAATAT AGGCTAAACC     450
AAGATAAAGT AATGTATTCA ACCTTTAGAA TTGTGCATTC ATAATTAGAT     500
CTTGTTTGTC GTAAAAAATT AGAAAATATA TTTACAGTAA TTTGGAATAC     550
AAAGCTAAGG GGGAAGTAAC TAATATTCTA GTGGAGGGAG GGACCAGTAC     600
CAGTACCTAG ATATTATTTT TAATTACTAT AATAATAATT TAATTAACAC     650
GAGACATAGG AATGTCAAGT GGTAGCGTAG GAGGGAGTTG GTTTAGTTTT     700
TTAGATACTA GGAGACAGAA CCGGACGGCC CATTGCAAGG CCAAGTTGAA     750
GTCCAGCCGT GAATCAACAA AGAGAGGGCC CATAATACTG TCGATGAGCA     800
TTTCCCTATA ATACAGTGTC CACAGTTGCC TTCTGCTAAG GGATAGCCAC     850
CCGCTATTCT CTTGACACGT GTCACTGAAA CCTGCTACAA ATAAGGCAGG     900
CACCTCCTCA TTCTCACTCA CTCACTCACA CAGCTCAACA AGTGGTAACT     950
TTTACTCATC TCCTCCAATT ATTTCTGATT TCATGCATGT TTCCCTACAT    1000
TCTATTATGA ATCGTGTTGT GGTGTATAAA CGTTGTTTCA TATCTCATCT    1050
CATCTATTCT GATTTTGATT CTCTTGCCTA CTGTAATCGG TGATAAATGT    1100
GAATGCTTCC TTTCTTCTCA GAAATCAATT TCTGTTTTGT TTTTGTTCAT    1150
CTGTAGCTTA TTCTCTGGTA GATTCCCCTT TTTGTAGACC ACACATCAC     1199
                                                          1241
ATG GCA AGC ATC ACA GCT TCA CAC CAC TTT GTG TCA AGA AGC
Met Ala Ser Ile Thr Ala Ser His His Phe Val Ser Arg Ser
 1              5                  10

                                                          1283
CAA ACT TCA CTA GAC ACC AAA TCA ACC TTG TCA CAG ATA GGA
Gln Thr Ser Leu Asp Thr Lys Ser Thr Leu Ser Gln Ile Gly
 15                 20                  25

                                                          1325
CTC AGG AAC CAT ACT CTG ACT CAC AAT GGT TTA AGG GCT GTT
Leu Arg Asn His Thr Leu Thr His Asn Gly Leu Arg Ala Val
     30                 35                  40

                                                          1367
AAC AAG CTT GAT GGG CTC CAA TCA ACA ACT AAT ACT AAG GTA
Asn Lys Leu Asp Gly Leu Gln Ser Thr Thr Asn Thr Lys Val
         45                 50                  55

                                                          1409
ACA CCC AAG ATG GCA TCC AGA ACT GAG ACC AAG AGA CCT GGA
Thr Pro Lys Met Ala Ser Arg Thr Glu Thr Lys Arg Pro Gly
             60                 65                  70

                                                          1451
TGC TCA GCT ACC ATT GTT TGT GGA AAG GGA ATG AAC TTG ATC
Cys Ser Ala Thr Ile Val Cys Gly Lys Gly Met Asn Leu Ile
                 75                 80

                                                          1493
TTT GTG GGT ACT GAG GTT GGT CCT TGG AGC AAA ACT GGT GGA
Phe Val Gly Thr Glu Val Gly Pro Trp Ser Lys Thr Gly Gly
 85                  90                 95
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CTA GGT GAT GTT CTT GGT GGA CTA CCA CCA GCC CTT GCA              1532
Leu Gly Asp Val Leu Gly Gly Leu Pro Pro Ala Leu Ala
    100                 105                 110

GTAAGTCTTT CTTTCATTTG GTTACCTACT CATTCATTAC TTATTTTGTT            1582
TAGTTAGTTT CTACTGCATC AGTCTTTTTA TCATTTAG GCC CGC GGA             1629
                                           Ala Arg Gly

CAT CGG GTA ATG ACA ATA TCC CCC CGT TAT GAC CAA TAC AAA          1671
His Arg Val Met Thr Ile Ser Pro Arg Tyr Asp Gln Tyr Lys
115                 120                 125

GAT GCT TGG GAT ACT GGC GTT GCG GTT GAG     GTACATCTTC           1711
Asp Ala Trp Asp Thr Gly Val Ala Val Glu
    130                 135

CTATATTGAT ACGGTACAAT ATTGTTCTCT TACATTTCCT GATTCAAGAA           1761
TGTGATCATC TGCAG GTC AAA GTT GGA GAC AGC ATT GAA ATT GTT         1806
                 Val Lys Val Gly Asp Ser Ile Glu Ile Val
                     140                 145

CGT TTC TTT CAC TGC TAT AAA CGT GGG GTT GAT CGT GTT TTT          1848
Arg Phe Phe His Cys Tyr Lys Arg Gly Val Asp Arg Val Phe
    150                 155                 160

GTT GAC CAC CCA ATG TTC TTG GAG AAA         GTAAGCATAT           1885
Val Asp His Pro Met Phe Leu Glu Lys
        165                 170

TATGATTATG AATCCGTCCT GAGGGATACG CAGAACAGGT CATTTTGAGT           1935
ATCTTTTAAC TCTACTGGTG CTTTTACTCT TTTAAG GTT TGG GGC AAA          1983
                                        Val Trp Gly Lys
                                                    175

ACT GGT TCA AAA ATC TAT GGC CCC AAA GCT GGA CTA GAT TAT          2025
Thr Gly Ser Lys Ile Tyr Gly Pro Lys Ala Gly Leu Asp Tyr
                180                 185

CTG GAC AAT GAA CTT AGG TTC AGC TTG TTG TGT CAA                  2061
Leu Asp Asn Glu Leu Arg Phe Ser Leu Leu Cys Gln
190                 195                 200

GTAAGTTAGT TACTCTTGAT TTTTATGTGG CATTTTACTC TTTTGTCTTT           2111
AATCGTTTTT TTAACCTTGT TTTCTCAG GCA GCC CTA GAG GCA CCT            2157
                               Ala Ala Leu Glu Ala Pro
                                                   205

AAA GTT TTG AAT TTG AAC AGT AGC AAC TAC TTC TCA GGA CCA          2199
Lys Val Leu Asn Leu Asn Ser Ser Asn Tyr Phe Ser Gly Pro
        210                 215                 220
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                  GTAATTAACA CATCCTAGTT TCAGAAAACT CCTTACTATA     2243
TAT G
Tyr G

TCATTGTAGG TAATCATCTT TATTTTGCCT ATTCCTGCAG GA GAG GAT          2291
                                            ly Glu Asp
                                                   225

GTT CTC TTC ATT GCC AAT GAT TGG CAC ACA GCT CTC ATT CCT          2333
Val Leu Phe Ile Ala Asn Asp Trp His Thr Ala Leu Ile Pro
                230                 235

TGC TAC TTG AAG TCA ATG TAC CAG TCC AGA GGA ATC TAC TTG          2375
Cys Tyr Leu Lys Ser Met Tyr Gln Ser Arg Gly Ile Tyr Leu
240                 245                 250

AAT GCC AAG           GTAAAATTTC TTTGTATTCA CTCGATTGCA           2414
Asn Ala Lys
    255

CGTTACCCTG CAAATCAGTA AGGTTGTATT AATATATGAT AAATTTCACA           2464
TTGCCTCCAG GTT GCT TTC TGC ATC CAT AAC ATT GCC TAC CAA           2507
           Val Ala Phe Cys Ile His Asn Ile Ala Tyr Gln
                       260                     265

GGT CGA TTT TCT TTC TCT GAC TTC CCT CTT CTC AAT CTT CCT          2549
Gly Arg Phe Ser Phe Ser Asp Phe Pro Leu Leu Asn Leu Pro
        270                 275                     280

GAT GAA TTC AGG GGT TCT TTT GAT TTC ATT GAT GGG TAT              2588
Asp Glu Phe Arg Gly Ser Phe Asp Phe Ile Asp Gly Tyr
            285                 290

GTATTTATGC TTGAAATCAG ACCTCCAACT TTTGAAGCTC TTTTGATGCT           2638
AGTAAATTGA GTTTTTAAAA TTTTGCAGAT ATGAG AAG CCT GTT AAG           2685
                                       Lys Pro Val Lys
                                       295

GGT AGG AAA ATC AAC TGG ATG AAG GCT GGG ATA TTA GAA TCA          2727
Gly Arg Lys Ile Asn Trp Met Lys Ala Gly Ile Leu Glu Ser
    300                 305                 310

CAT AGG GTG GTT ACA GTG AGC CCA TAC TAT GCC CAA GAA CTT          2769
His Arg Val Val Thr Val Ser Pro Tyr Tyr Ala Gln Glu Leu
        315                 320                 325

GTC TCT GCT GTT GAC AAG GGA GTT GAA TTG GAC AGT GTC CTT          2811
Val Ser Ala Val Asp Lys Gly Val Glu Leu Asp Ser Val Leu
            330                 335                 340

CGT AAG ACT TGC ATA ACT GGG ATT GTG AAT GGC ATG GAT ACA          2853
Arg Lys Thr Cys Ile Thr Gly Ile Val Asn Gly Met Asp Thr
                345                 350
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CAA GAG TGG AAC CCA GCG ACT GAC AAA TAC ACA GAT GTC AAA 2895
Gln Glu Trp Asn Pro Ala Thr Asp Lys Tyr Thr Asp Val Lys
355 360 365

TAC GAT ATA ACC ACT GTAAGATAAG ATTTTTCCGA CTCCAGTATA 2940
Tyr Asp Ile Thr Thr
370

TACTAAATTA TTTTGTATGT TTATGAAATT AAAGAGTTCT TGCTAATCAA 2990
AATCTCTATA CAG GTC ATG GAC GCA AAA CCT TTA CTA AAG GAG 3033
Val Met Asp Ala Lys Pro Leu Leu Lys Glu
375 380

GCT CTT CAA GCA GCA GTT GGC TTG CCT GTT GAC AAG AAG ATC 3075
Ala Leu Gln Ala Ala Val Gly Leu Pro Val Asp Lys Lys Ile
385 390 395

CCT TTG ATT GGC TTC ATC GGC AGA CTT GAG GAG CAG AAA GGT 3117
Pro Leu Ile Gly Phe Ile Gly Arg Leu Glu Glu Gln Lys Gly
400 405 410

TCA GAT ATT CTT GTT GCT GCA ATT CAC AAG TTC ATC GGA TTG 3159
Ser Asp Ile Leu Ala Val Ala Ile His Lys Phe Ile Gly Leu
415 420 425

GAT GTT CAA ATT GTA GTC CTT GTAAGTACCA AATGGACTCA 3200
Asp Val Gln Ile Val Val Leu
430

TGGTATCTCT CTTGTTGAGT TTACTTGTGC CGAAACTGAA ATTGACCTGC 3250
TACTCATCCT ATGCATCAG GGA ACT GGC AAA AAG GAG TTT GAG 3293
Gly Thr Gly Lys Lys Glu Phe Glu
435 440

CAG GAG ATT GAA CAG CTC GAA GTG TTG TAC CCT AAC AAA GCT 3335
Gln Glu Ile Glu Gln Leu Glu Val Leu Tyr Pro Asn Lys Ala
445 450

AAA GGA GTG GCA AAA TTC AAT GTC CCT TTG GCT CAC ATG ATC 3377
Lys Gly Val Ala Lys Phe Asn Val Pro Leu Ala His Met Ile
455 460 465

ACT GCT GGT GCT GAT TTT ATG TTG GTT CCA AGC AGA TTT GAA 3419
Thr Ala Gly Ala Asp Phe Met Leu Val Pro Ser Arg Phe Glu
470 475 480

CCT TGT GGT CTC ATT CAG TTA CAT GCT ATG CGA TAT GGA ACA 3461
Pro Cys Gly Leu Ile Gln Leu His Ala Met Arg Tyr Gly Thr
485 490 495

GTAAGAACCA GAAGAGCTTG TACCTTTTTA CTGAGTTTTT AAAAAAAGAA 3511
TCATAAGACC TTGTTTTCCA TCTAAAGTTT AATAACCAAC TAAATGTTAC 3561
TGCAGCAAGC TTTTCATTTC TGAAAATTGG TTATCTGATT TTAACGTAAT 3611

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CACATGTGAG TCAG GTA CCA ATC TGT GCA TCG ACT GGT GGA CTT     3655
                Val Pro Ile Cys Ala Ser Thr Gly Gly Leu
                        500                     505

GTT GAC ACT GTG AAA GAA GGC TAT ACT GGA TTC CAT ATG GGA     369?
Val Asp Thr Val Lys Glu Gly Tyr Thr Gly Phe His Met Gly
            510                 515                 520

GCC TTC AAT GTT GAA    GTATGTGATT TTACATCAAT TGTGTACTTG     3742
Ala Phe Asn Val Glu
                525

TACATGGTCC ATTCTCGTCT TGATATACCC CTTGTTGCAT AAACATTAAC      3792
TTATTGCTTC TTGAATTTGG TTAG TGC GAT GTT GTT GAC CCA GCT      3837
                           Cys Asp Val Val Asp Pro Ala
                                           530

GAT GTG CTT AAG ATA GTA ACA ACA GTT GCT AGA GCT CTT GCA     3879
Asp Val Leu Lys Ile Val Thr Thr Val Ala Arg Ala Leu Ala
        535                 540                 545

GTC TAT GGC ACC CTC GCA TTT GCT GAG ATG ATA AAA AAT TGC     3921
Val Tyr Gly Thr Leu Ala Phe Ala Glu Met Ile Lys Asn Cys
            550                 555                 560

ATG TCA GAG GAG CTC TCC TGG AAG   GTAAGTGTGA ATTTGATAAT     3965
Met Ser Glu Glu Leu Ser Trp Lys
                565

TTGCGTAGGT ACTTCAGTTT GTTGTTCTCG TCAGCACTGA TGGATTCCAA      40??
CTGGTGTTCT TGCAG      GAA CCT GCC AAG AAA TGG GAG ACA TTG   40??
                      Glu Pro Ala Lys Lys Trp Glu Thr Leu
                          570                     575

CTA TTG GGC TTA GGA GCT TCT GGC AGT GAA CCC GGT GTT GAA     4099
Leu Leu Gly Leu Gly Ala Ser Gly Ser Glu Pro Gly Val Glu
        580                 585                 590

GGG GAA GAA ATC GCT CCA CTT GCC AAG GAA AAT GTA GCC ACT     4141
Gly Glu Glu Ile Ala Pro Leu Ala Lys Glu Asn Val Ala Thr
            595                 600                     605

CCT TAA     ATGAGCTTTG GTTATCCTTG TTTCAACAAT AAGATCATTA     4187
Pro ***
606

AGCAAACGTA TTTACTAGCG AACTATGTAG AACCCTATTA TGGGGTCTCA      4237
ATCATCTACA AAATGATTGG TTTTTGCTGG GGAGCAGCAG CATATAAGGC      4287
TGTAAAATCC TGGTTAATGT TTTTGTAGGT AAGGGCTATT TAAGGTGGTG      4337
TGGATCAAAG TCAATAGAAA ATAGTTATTA CTAACGTTTG CAACTAAATA      4387
CTTAGTAATG TAGCATAAAT AATACTAGAA CTAGTAGCTA ATATATATGC      4437
GTGAATTTGT TGTACCTTTT CTTGCATAAT TATTTGCAGT ACATATATAA      4487
TGAAAATTAC CCAAGGAATC AATGTTTCTT GCTCCGTCCT CCTTTGATGA      4537
TTTTTTACCC AATACAGAGC TAGTGTGTTA TGTTATAAAT TTTGTTTAAA      4587
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AGAAGTAATC AAATTCAAAT TAGTTGTTTG GTCATATGAA AGAAGCTGCC 4637
AGGCTAACTT TGAGGAGATG GCTATTGAAT TTCAAAATGA TTATGTGAAA 4687
ACAATGCAAC ATCTATGTCA ATCAACACTT AAATTATTGC ATTTAGAAAG 4737
ATATTTTTGA GCCCATGACA CATTCATTCA TAAAGTAAGG TAGTATGTAT 4787
GATTGAATGG ACTACAGCTC AATCAAAGCA TCTCCTTTAC ATAACGGCAC 4837
TGTCTCTTGT CTACTACTCT ATTGGTAGTA GTAGTAGTAA TTTTACAATC 4887
CAAATTGAAT AGTAATAAGA TGCTCTCTAT TTACTAAAGT AGTAGTATTA 4937
TTCTTTCGTT ACTCTAAAGC AACAAAA 4964

CLAIMS

1. Method of suppressing amylose formation in potato, characterised by genetically engineered modification of the potato by introducing into the genome of the potato tissue a gene construct comprising a fragment of the potato gene which codes for formation of granule-bound starch synthase (GBSS gene) inserted in the antisense direction, said fragment being selected among the fragments which essentially have the nucleotide sequences stated in SEQ ID No. 1, SEQ ID No. 2 and SEQ ID No. 3 together with a promoter selected among CaMV 35S, patatin I and the GBSS promoter.

2. Amylopectin-type native starch, characterised in that it has been obtained from potato which has been modified in a genetically engineered manner for suppressing formation of amylose-type starch.

3. Derivatised amylopectin-type starch, characterised in that it is amylopectin-type starch extracted from potato which has been modified in a genetically engineered manner for suppressing formation of amylose-type starch, said amylopectin-type starch subsequently being derivatised in a chemical, physical or enzymatic manner.

4. Fragment of the gene coding for granule-bound starch synthase (GBSS) in potato, said fragment being selected among the fragments which essentially have the nucleotide sequences stated in SEQ ID No. 1, SEQ ID No. 2 and SEQ ID No. 3.

5. Promoter for the gene for granule-bound starch synthase (GBSS) in potato, said promoter being tuber-specific and having essentially the nucleotide sequence stated in SEQ ID No. 4.

6. Gene coding for granule-bound starch synthase in potato (GBSS gene) having essentially the nucleotide sequence stated in SEQ ID No. 5.

7. Antisense construct for inhibiting expression of the gene for granule-bound starch synthase in potato, comprising

a) a promoter,

b) a fragment of the gene coding for granule-bound starch synthase inserted in the antisense direction, said fragment being selected among the fragments having essentially the nucleotide sequences stated in SEQ ID No. 1, SEQ ID No. 2 and SEQ ID No. 3.

8. Antisense construct as claimed in claim 7, characterised in that the promoter essentially has the sequence stated in SEQ ID No. 4.

9. Antisense construct as claimed in claim 7, characterised in that the promoter is selected among the CaMV 35S promoter and the patatin I promoter.

10. Vector comprising a fragment of the gene coding for granule-bound starch synthase (GBSS) in potato, said fragment being selected among the fragments having essentially the nucleotide sequences stated in SEQ ID No. 1, SEQ ID No. 2 and SEQ ID No. 3, and inserted in the antisense direction.

11. Vector comprising the antisense construct as claimed in any one of claims 7-9.

12. Cell of potato plant whose genome comprises the antisense construct as claimed in any one of claims 7-9.

13. Potato plant whose genome comprises the antisense construct as claimed in any one of claims 7-9.

14. Potato tubers whose genome comprises the antisense construct as claimed in any one of claims 7-9.

15. Seeds from potato plant, whose genome comprises the antisense construct as claimed in any one of claims 7-9.

16. Microtubers of potato, whose genome comprises the antisense construct as claimed in any one of claims 7-9.

FIG.1

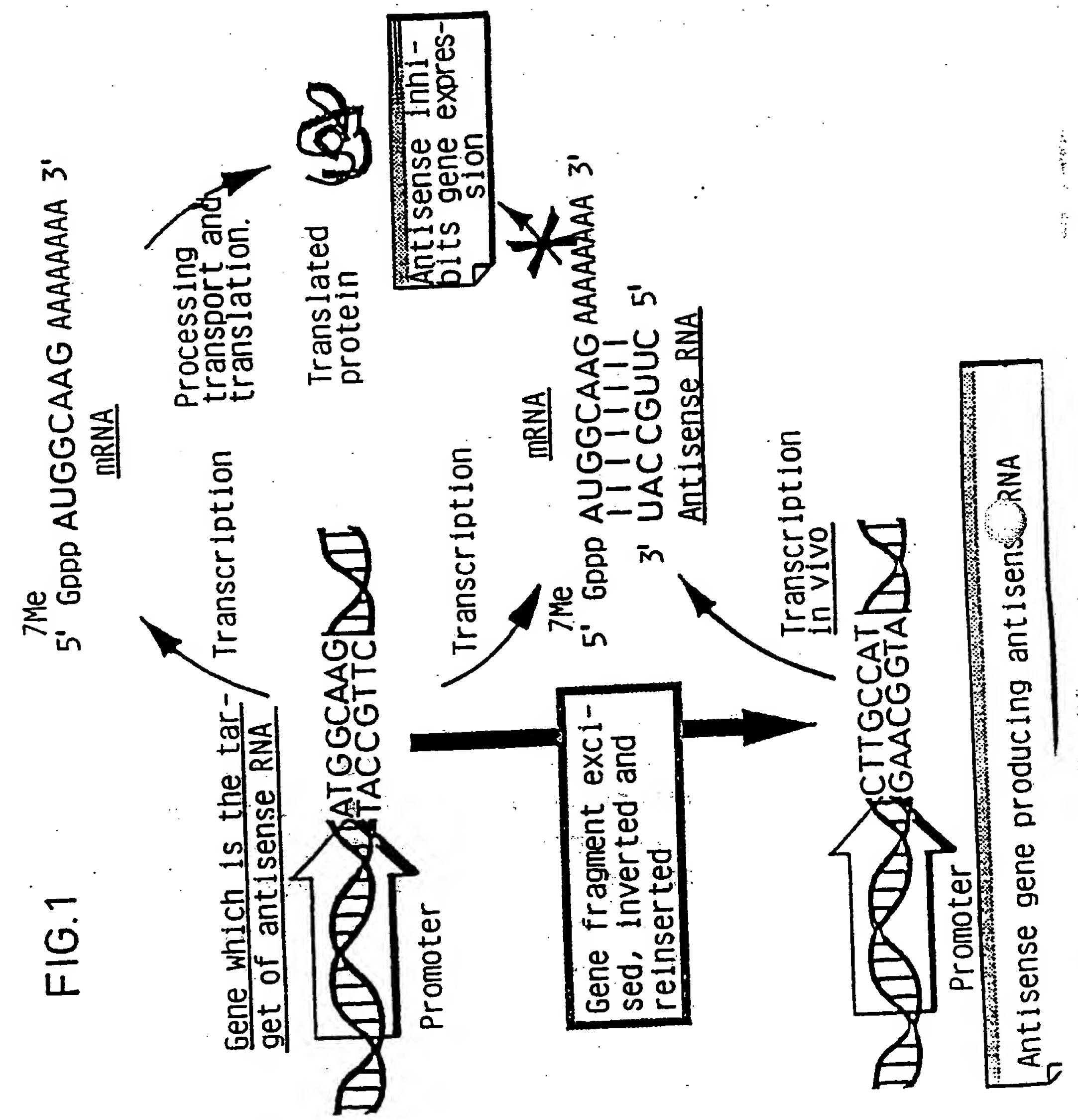
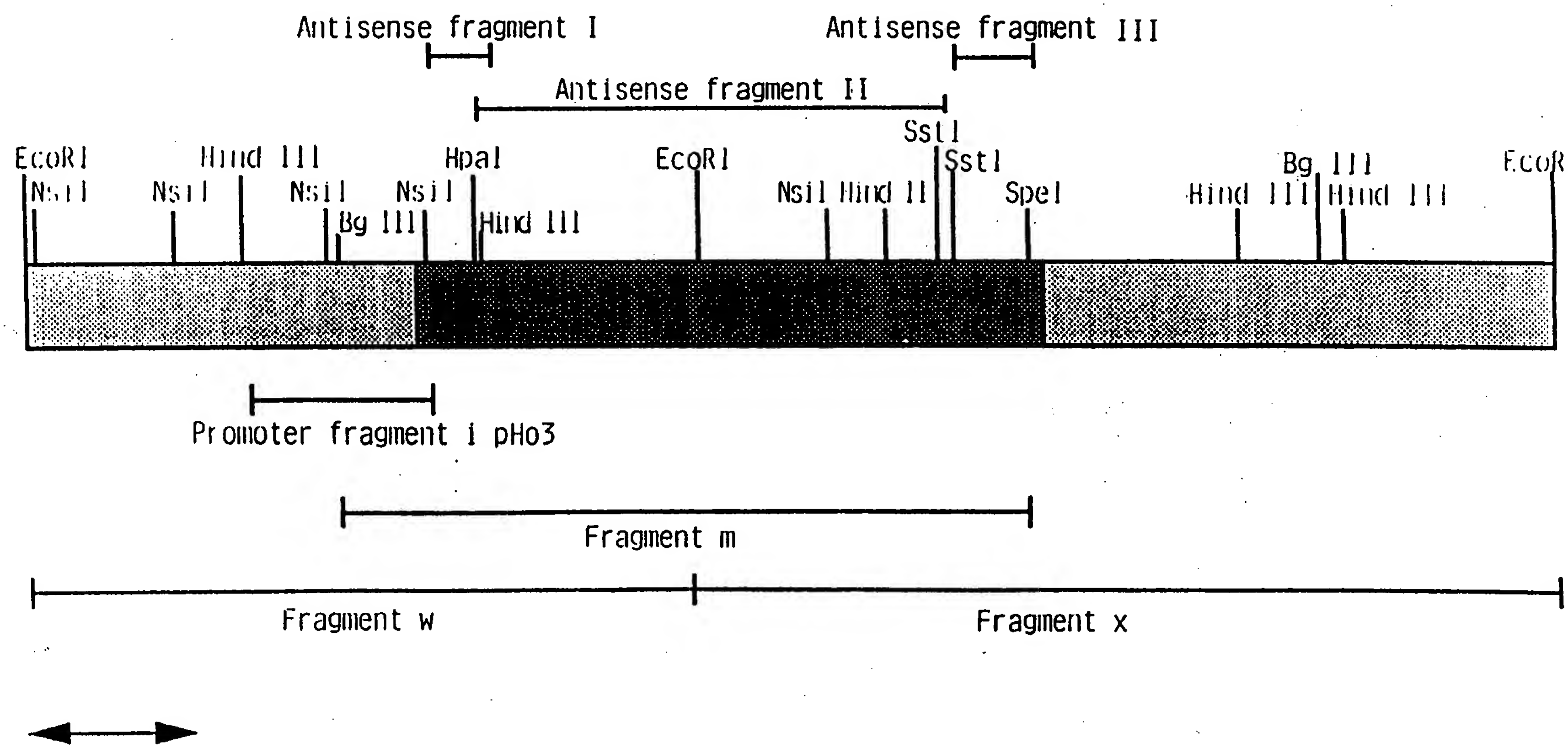

7Me
5' Gppp AUGGCAAG AAAAAAA 3'
mRNA
Transcription
Processing
transport and
translation.
Translated
protein
Antisense inhi-
bits gene expres-
sion
Gene which is the tar-
get of antisense RNA
ATGGCAAG
TACCGTTC
Promoter
Gene fragment exci-
sed, inverted and
reinserted
Transcription
7Me
5' Gppp AUGGCAAG AAAAAAA 3'
mRNA
3' UACCGUUC 5'
Antisense RNA
Transcription
in vivo
CTTGCCAT
GAACGGTA
Promoter
Antisense gene producing antisense RNA

FIG. 2 Result of restriction analysis. GBSS coding region including introns are marked in a darker tone.



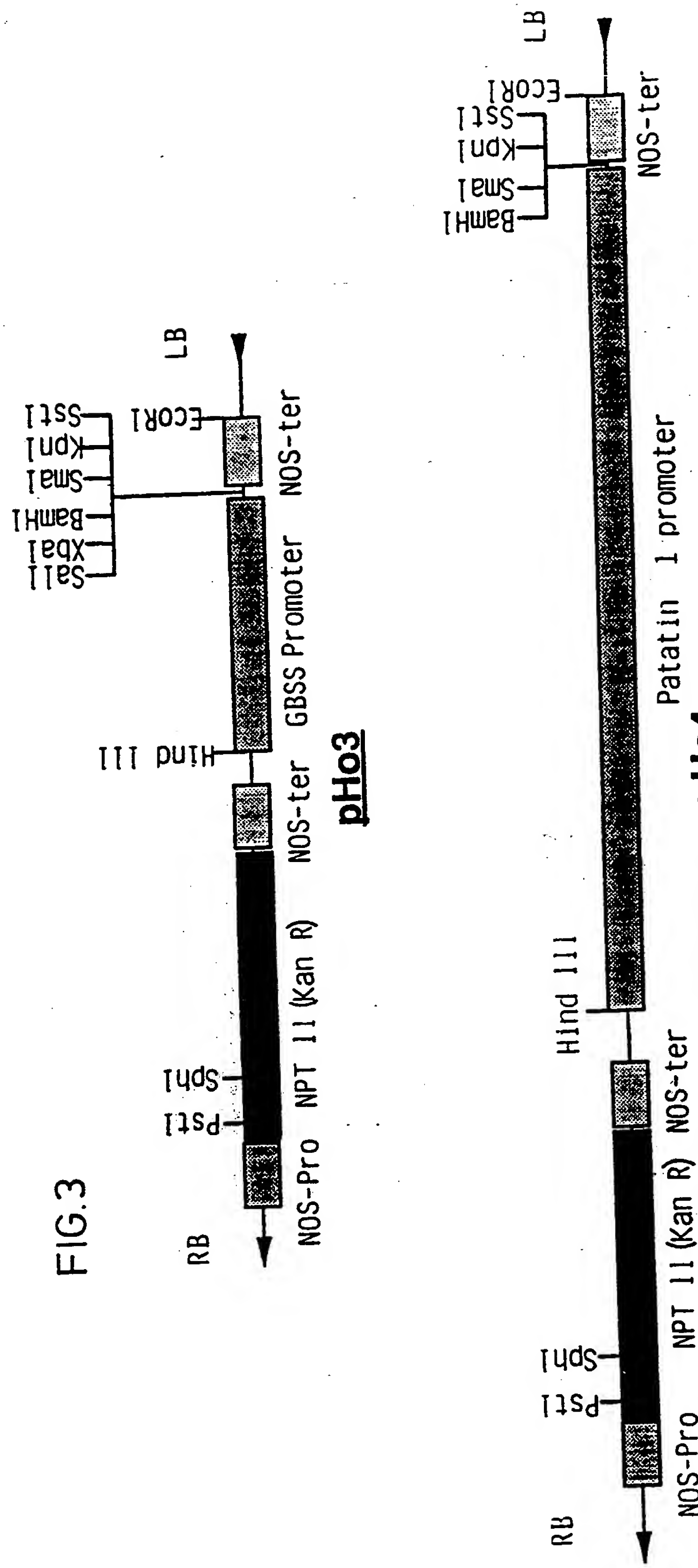

LB
SstI
KpnI
SmaI
BamHI
XbaI
SalI
EcoRI
NOS-ter
GBSS Promoter
Hind III
NOS-ter
NPT II (Kan R)
SphI
PstI
NOS-Pro
RB
pHo3
LB
EcoRI
SstI
KpnI
SmaI
BamHI
NOS-ter
Patatin 1 promoter
Hind III
NOS-ter
NPT II (Kan R)
SphI
PstI
NOS-Pro
RB
pHo4

FIG.3

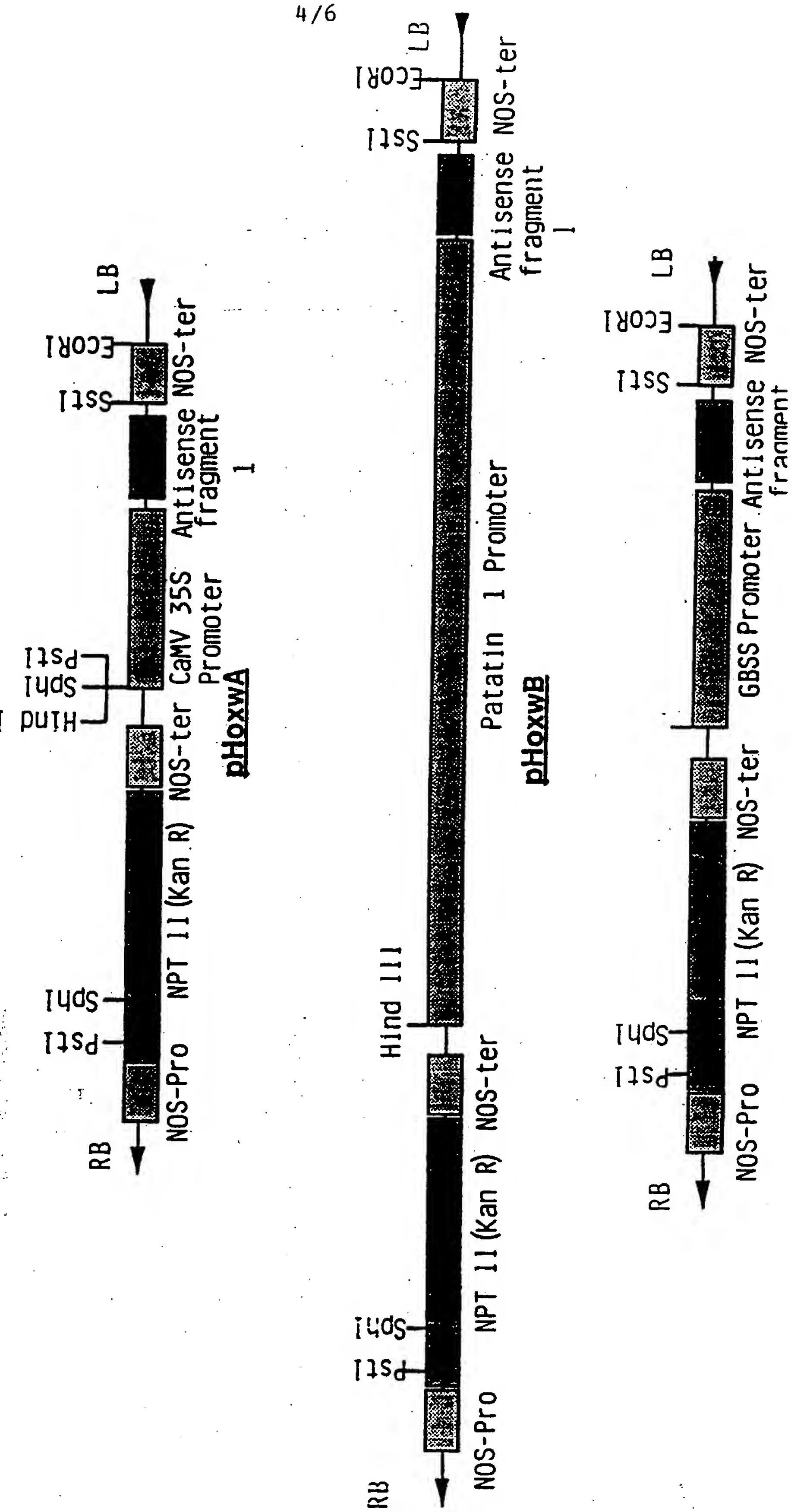

pHoxwA
RB
NOS-Pro
PstI
SphI
NPT II (Kan R)
NOS-ter
Hind III
SphI
PstI
CaMV 35S Promoter
Antisense fragment 1
SstI
NOS-ter
EcoRI
LB
pHoxwB
RB
NOS-Pro
PstI
SphI
NPT II (Kan R)
NOS-ter
Hind III
Patatin 1 Promoter
Antisense fragment 1
SstI
NOS-ter
EcoRI
LB
RB
NOS-Pro
PstI
SphI
NPT II (Kan R)
NOS-ter
GBSS Promoter
Antisense fragment
SstI
NOS-ter
EcoRI
LB

FIG. 4

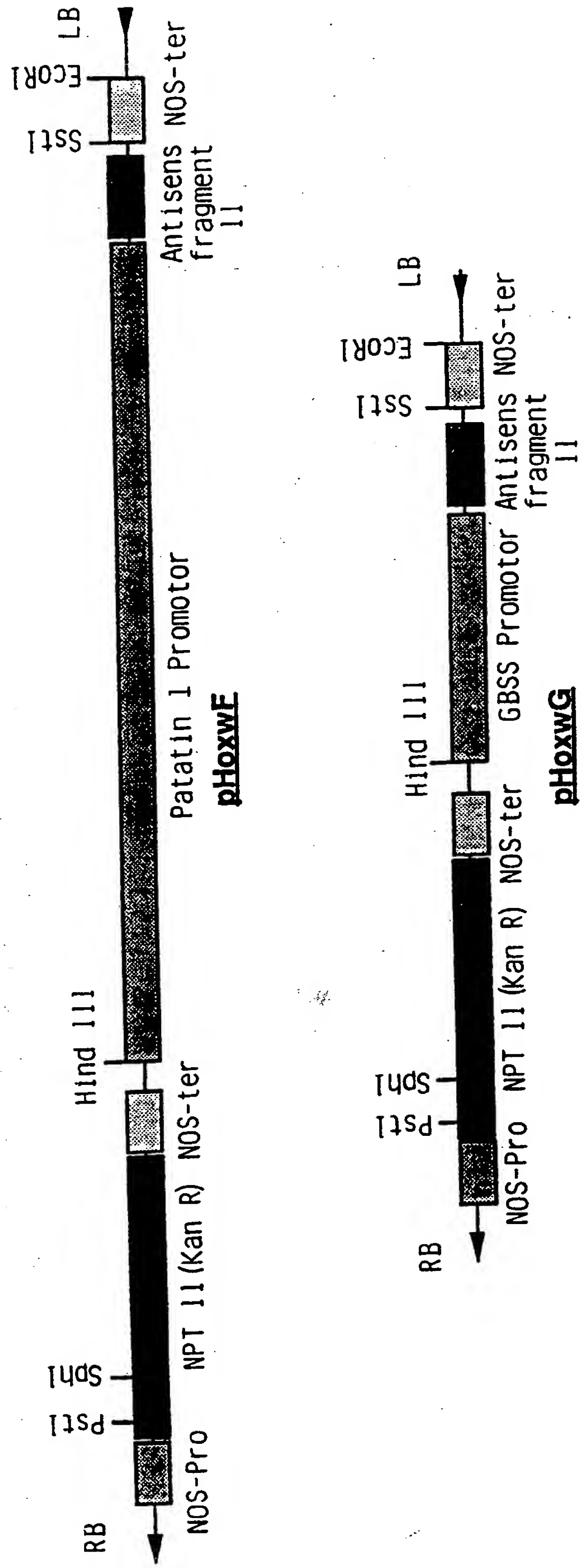
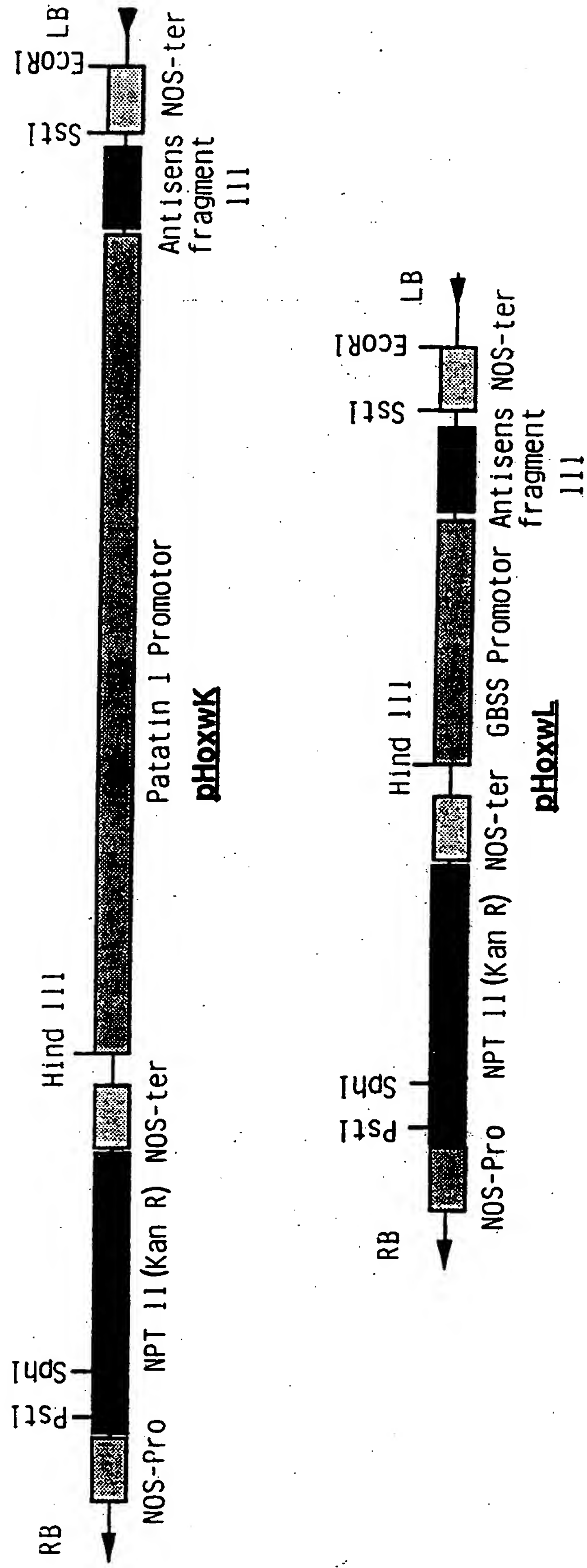

LB
EcoRI
SstI
NOS-ter
Antisens fragment II
Patatin I Promotor
pHoxwE
Hind III
NOS-ter
NPT II (Kan R)
SphI
PstI
NOS-Pro
RB
LB
EcoRI
SstI
NOS-ter
Antisens fragment II
GBSS Promotor
Hind III
pHoxwG
NOS-ter
NPT II (Kan R)
SphI
PstI
NOS-Pro
RB

FIG.5



LB
EcoRI
SstI
NOS-ter
Antisens fragment III
Patatin I Promotor
pHoxwK
Hind III
NOS-ter
NPT II (Kan R)
SphI
PstI
NOS-Pro
RB
LB
EcoRI
SstI
NOS-ter
Antisens fragment III
GBSS Promotor
Hind III
pHoxwL
NOS-ter
NPT II (Kan R)
SphI
PstI
NOS-Pro
RB

FIG.6

INTERNATIONAL SEARCH REPORT

International Application No PCT/SE 91/00892

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC5: C 12 N 15/56, 9/42, A 01 H 5/00

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
IPC5	C 12 N; A 01 H

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in Fields Searched[8]

SE,DK,FI,NO classes as above

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category *	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
P,X	MOL GEN GENET, Vol. 225, 1991 R.G.F. Visser et al: "Inhibition of the expression of the gene for granule-bound starch synthase in potato by antisense constructs", see page 289 - page 296 --	1-16
A	EP, A2, 0368506 (IMPERIAL CHEMICAL INDUSTRIES PLC) 16 May 1990, see especially claim 14 --	1-16
A	PLANT SCIENCE, Vol. 64, 1989 R.G.F. Visser et al: "Molecular cloning and partial characterization of the gene for granule-bound starch synthase from a wildtype and an amylose-free potato(solanum tuberosuml.)", cited in the application --	1-16

* Special categories of cited documents:[10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance, the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance, the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
30th March 1992	1992 -04- 01
International Searching Authority	**Signature of Authorized Officer**
SWEDISH PATENT OFFICE	Mikael G:son Bergstrand

Form PCT/ISA/210 (second sheet) (January 1985)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
A	EP, A2, 0335451 (VERENIGING VOOR CHRISTELIJK WETENSCHAPPELIJK ONDERWIJS) 4 October 1989, see the whole document --	1-16

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.PCT/SE 91/00892

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the Swedish Patent Office EDP file on 28/02/92
The Swedish Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A2- 0368506	90-05-16	AU-D-	4430789	90-08-16
		JP-A-	2273177	90-11-07
EP-A2- 0335451	89-10-04	JP-A-	2016985	90-01-19
		NL-A-	8800756	89-10-16

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